

Development of Translational Pharmacokinetic Model for Characterizing and Predicting Protein Therapeutics Disposition in Brain

Dhaval K. Shah, Ph.D.

dshah4@buffalo.edu

 **University at Buffalo** *The State University of New York*
School of Pharmacy and Pharmaceutical Sciences

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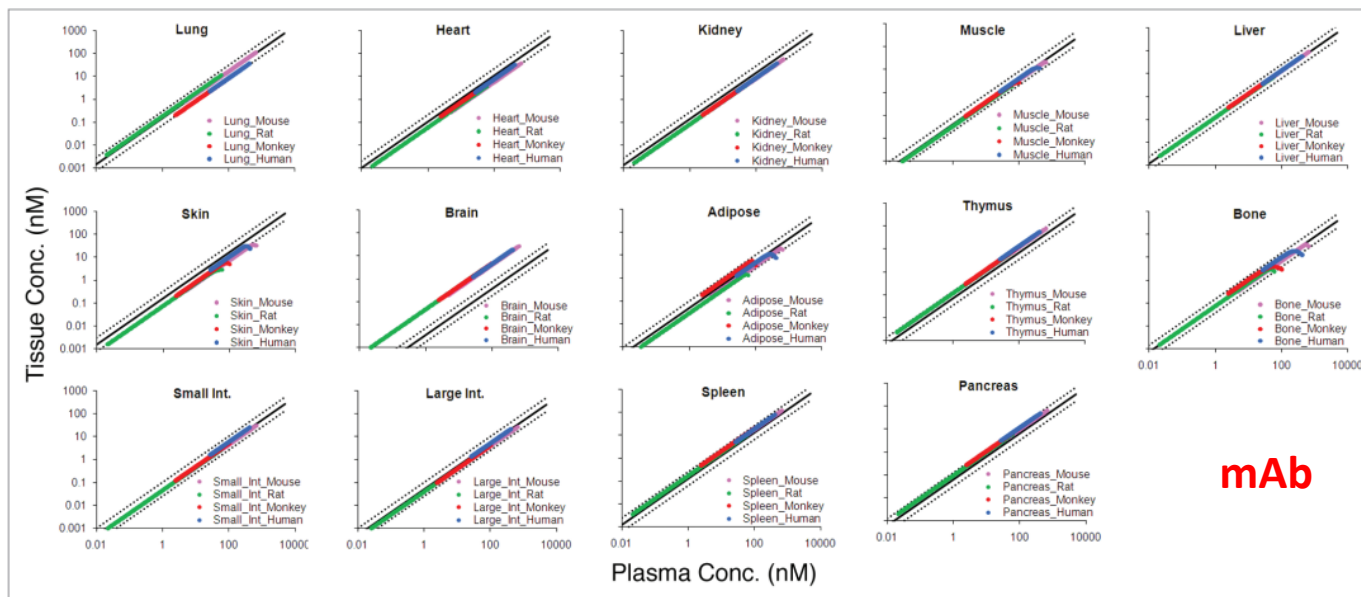
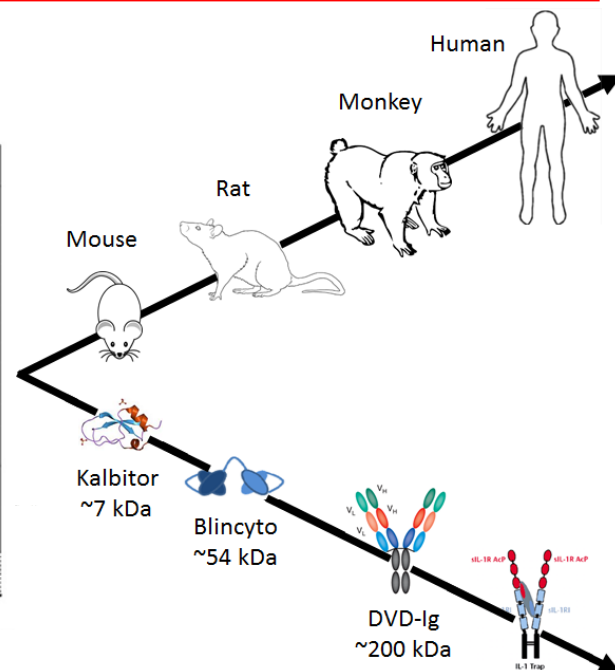
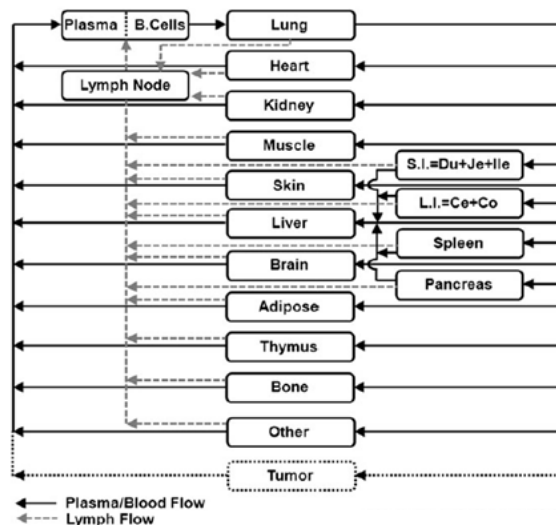
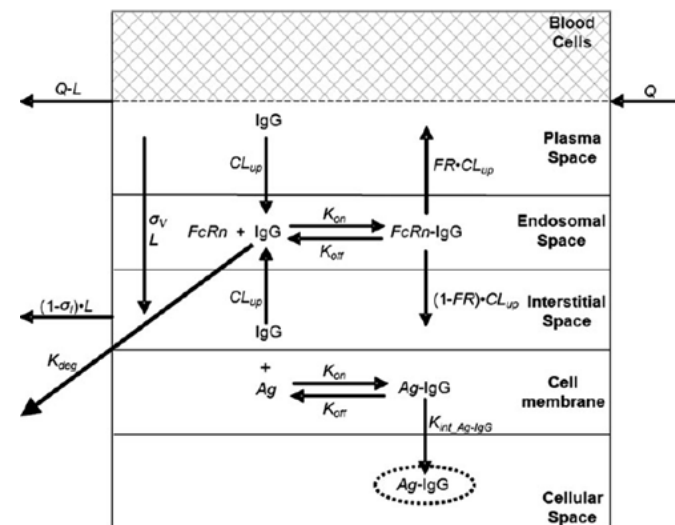
Forum on
NEUROSCIENCE and
NERVOUS SYSTEM DISORDERS

9/8/2017

Physiologically Based Pharmacokinetic Model

Shah et al. J Pharmacokinet Pharmacodyn (2012) 39:67–86

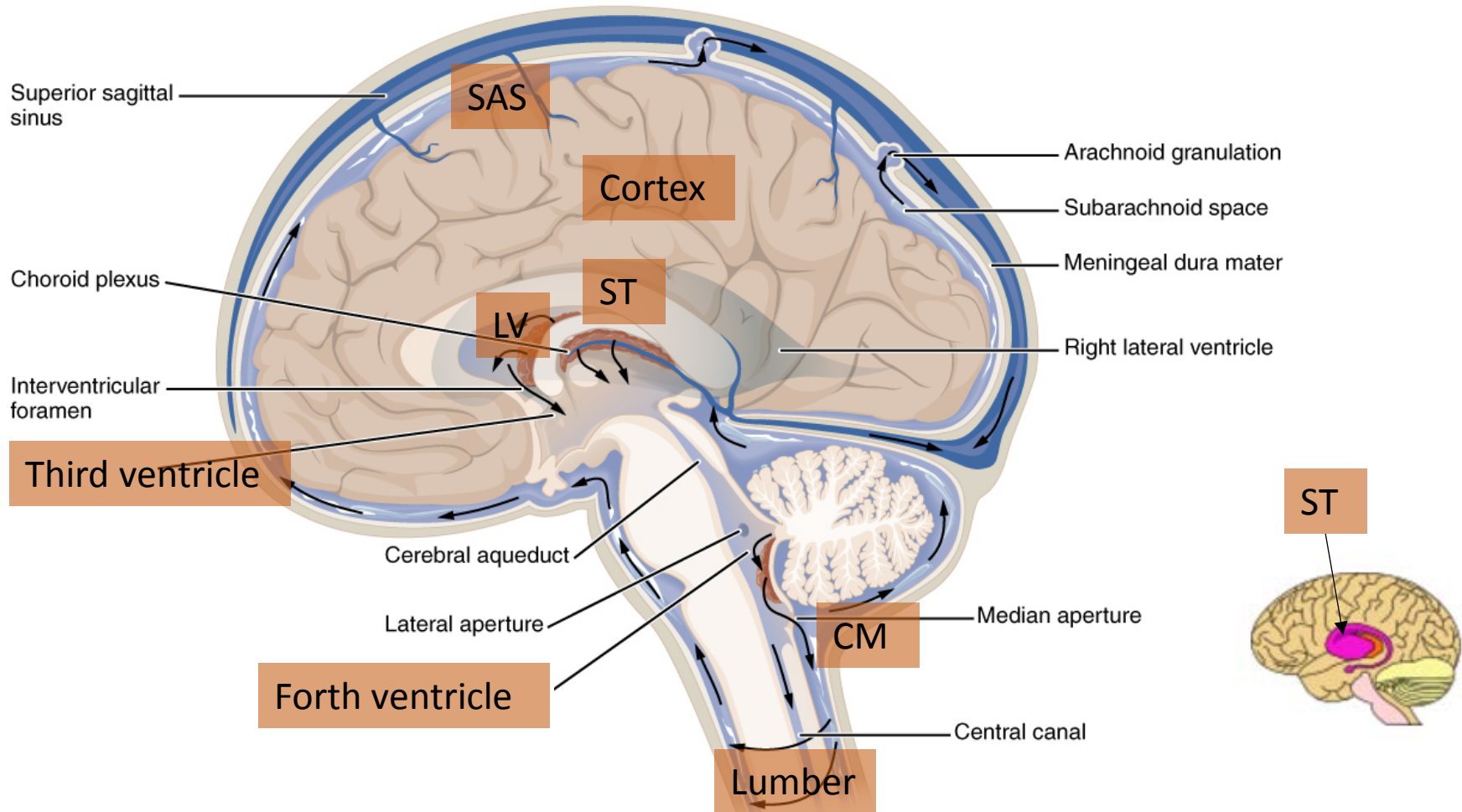
Shah et al. mAbs (2013) 5(2):297–305



	ABC (%)
Lung	14.9
Heart	10.2
Kidney	13.7
Muscle	3.97
Skin	15.7
Small Int.	5.22
Large Int.	5.03
Spleen	12.8
Liver	12.1
Bone	7.27
Stomach	4.98
Lymph nodes	8.46
Fat	4.78
Brain	0.351
Pancreas	6.40
Testes	5.88
Thyroid	67.5
Thymus	6.62

mAb

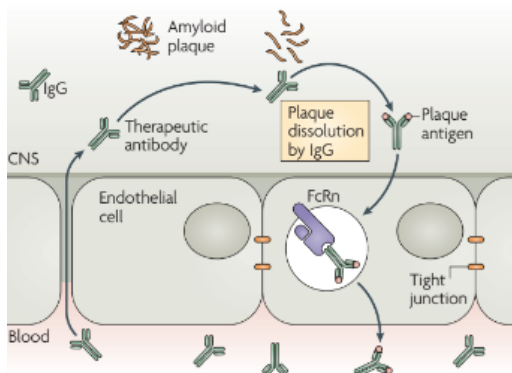
Complexity of Brain Anatomy and Physiology



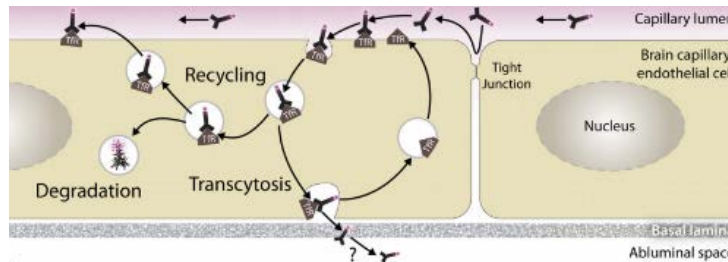
- Brain contains several distinct yet connected anatomical compartments
- The origin and the direction of CSF and ISF(ECF) bulk flow is still controversial
- Brain possesses unique (g)lymphatic system

Unknowns: Protein Therapeutics Disposition in Brain

FcRn

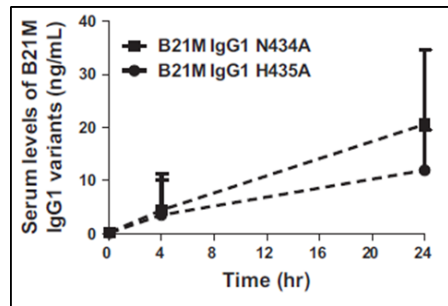
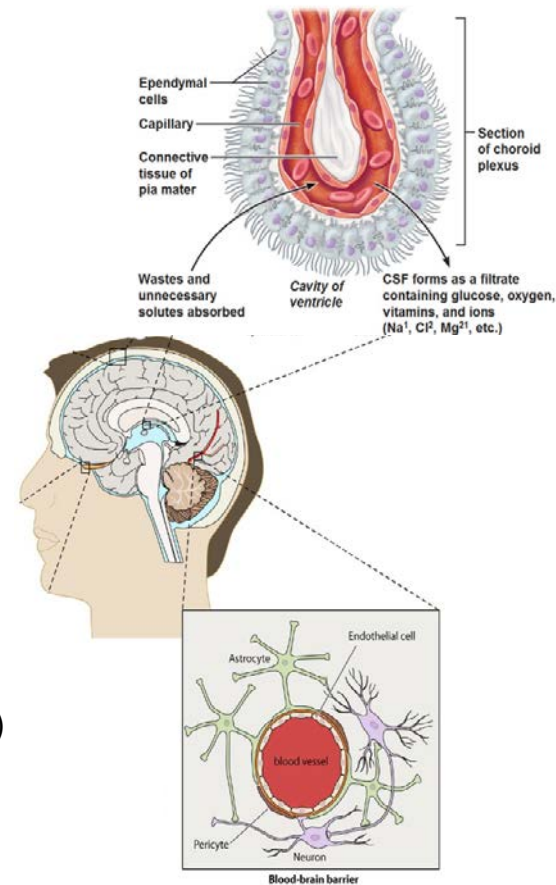


RMT Efficiency

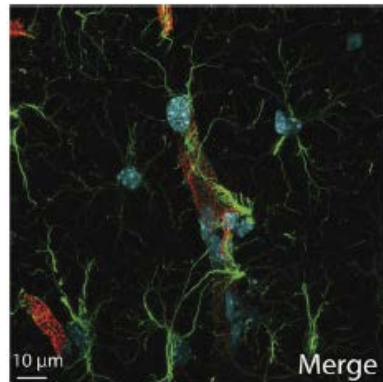
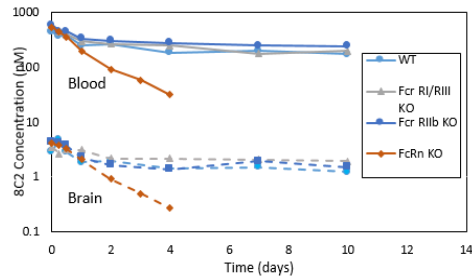


BBB vs BCSFB

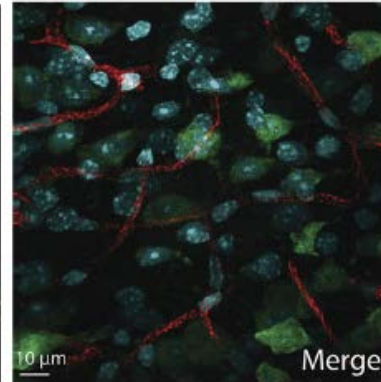
Cerebrospinal Fluid (CSF) – Choroid plexus



PK of 8C2, a murine monoclonal IgG1 Ab



neuronal marker NeuN (green) AF647-labeled anti-TfR MAbs Ri7 (red)

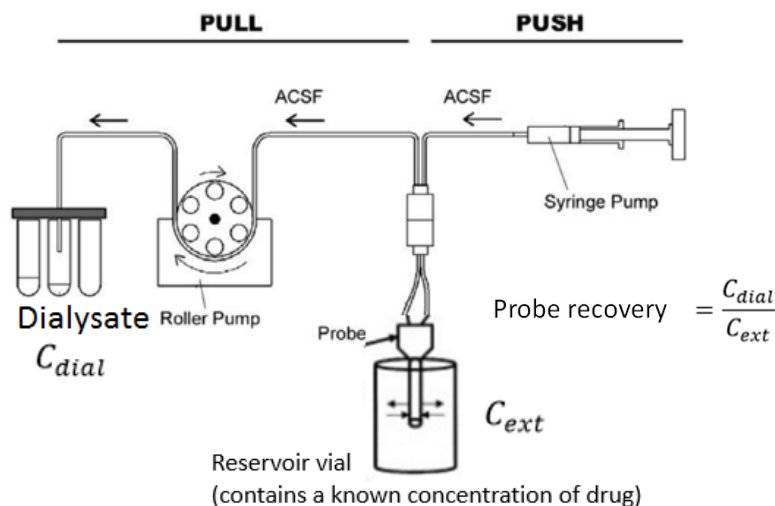
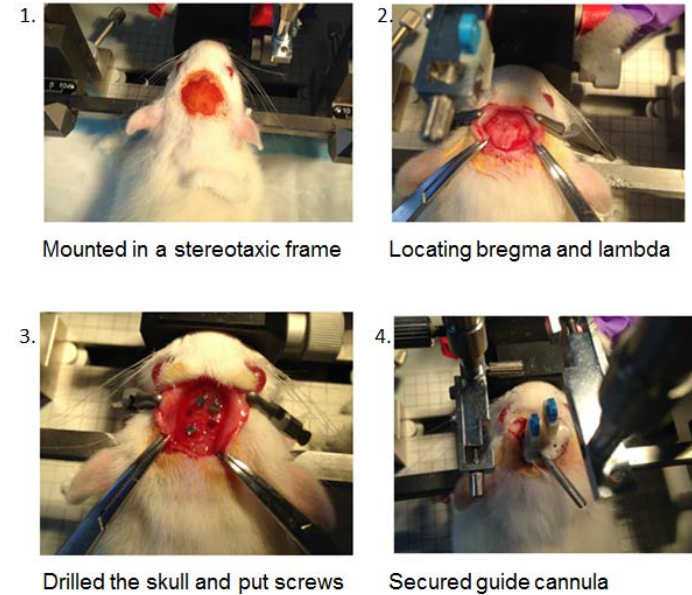
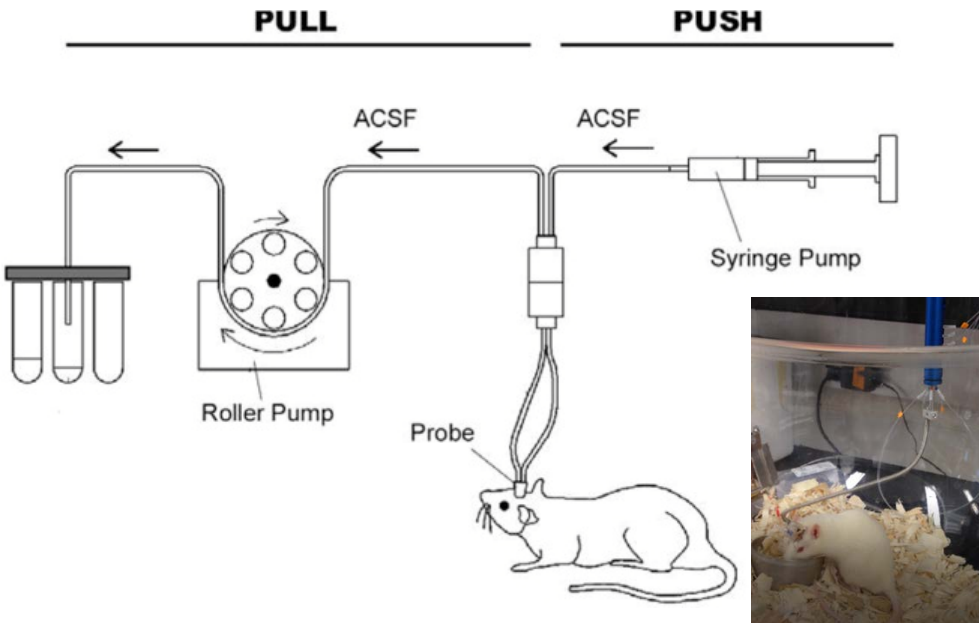


GFAP-labeled astrocytes (green) AF647-labeled anti-TfR MAbs Ri7 (red)

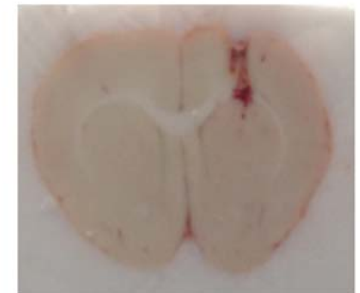
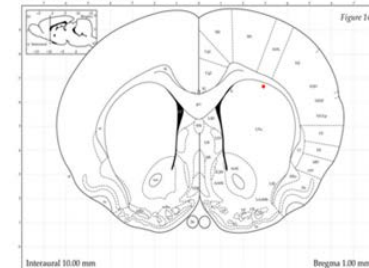
Overall Objectives

- ❑ Perform novel in vitro and in vivo experiments to understand and quantify the main determinants for protein therapeutics disposition in brain
- ❑ Develop a translational mathematical model that can simultaneously characterize in-house and published brain disposition data from diverse protein therapeutics in various animal species
- ❑ Use the mathematical model to quantitatively understand the main pathways of protein therapeutics entry into the brain and exit from the brain
- ❑ Design novel protein therapeutics that can exploit the major pathways of entry into the brain to cure various CNS disorders (e.g. glioblastoma and Alzheimer's disease)

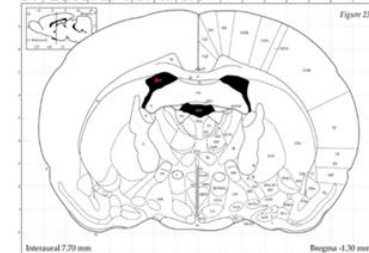
Microdialysis System for MAbs: Rat Brain



A. Striatum



B. Lateral Ventricle



Brain Disposition of Endogenous Ab

Rat endogenous IgG concentration (S.D.)			sample/plasma ratio
Rat Plasma (n=10)	1168 (650)	µg/mL	
Lateral ventricle (n=5)	2.26 (0.74)	µg/mL	0.0018 (500 fold)
Striatum (n=8)	3.2 (2.6)	µg/mL	0.003 (300 fold)
Cisterna Magna (n=10)	10.7 (8.25)	µg/mL	0.009 (100 fold)
Whole Brain (n=16)	1.68 (1.4)	µg/g	0.0014 (700 fold)

Case	Aβ ₁₋₄₂ pg/ml	T-tau pg/ml
<i>Brain interstitial fluid</i>		
1	129	1403
2	147	1611
3	79	1416
4	753	4023
5	346	9886
6	860	—
7	279	979
8	1495	—
9	272	3241
10	1727	209
11	37	1943
Mean±SD	557 ± 586	2746 ± 2914*
<i>Ventricular CSF</i>		
1	379	317
2	888	1237
3	348	2301
4	783	1211
5	780	1734
6	783	669
7	469	224
8	441	3652
9	894	362
10	167	116
11	413	78
Mean±SD	577 ± 253	1082 ± 1118

Comparison with Aβ as an endogenous protein

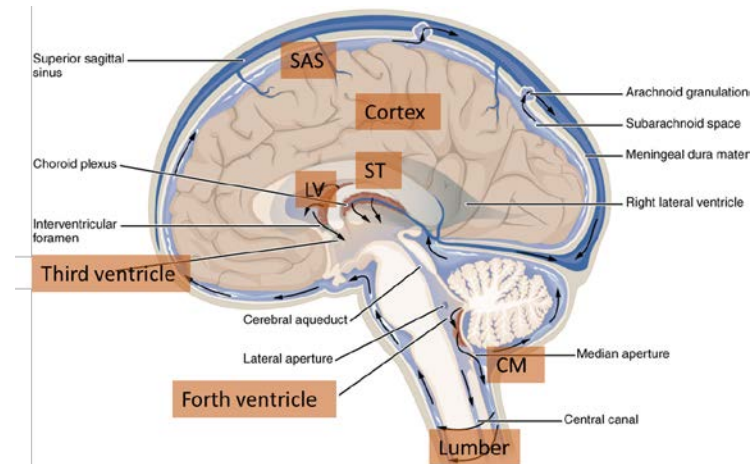
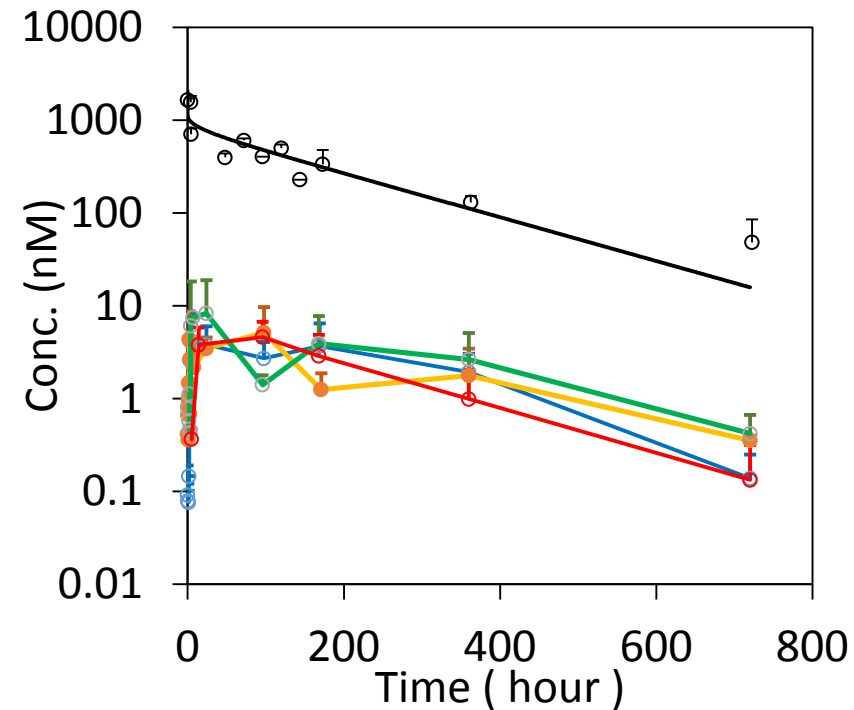
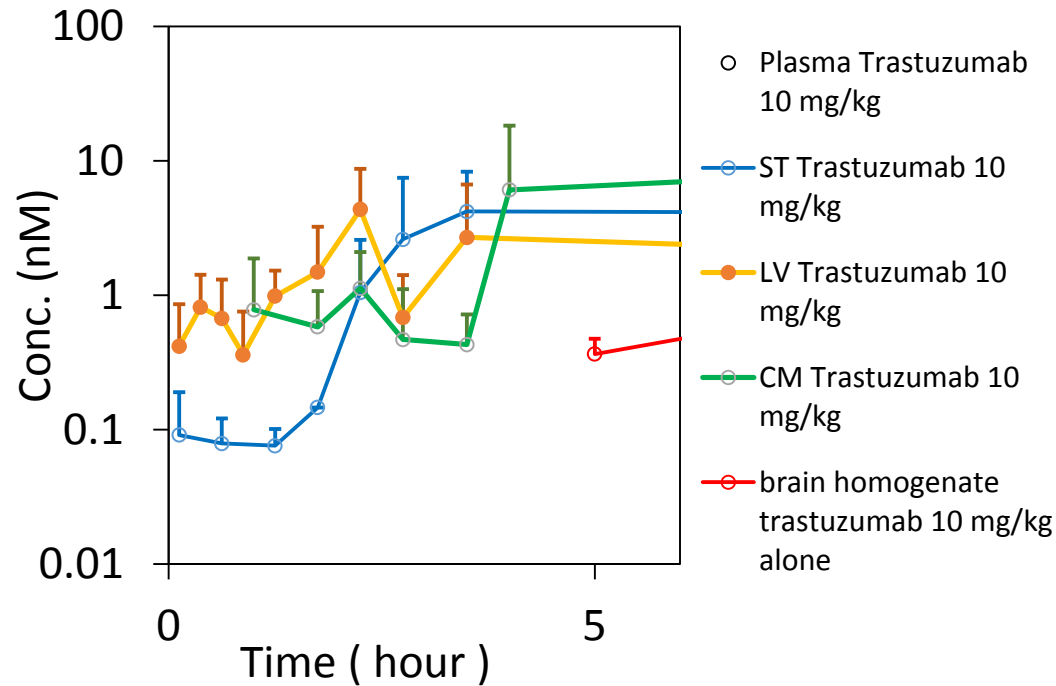


Table 1. Concentrations of Aβ in the ISF, CSF, and tissue lysates in young and aged mice

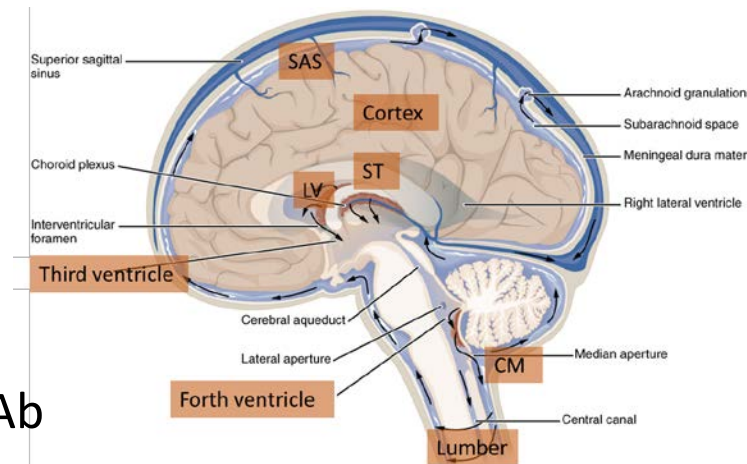
Tissue-fluid	Mean ± SEM concentration					
	3 months			12–15 months		
	Aβ _{1-x}	Aβ ₄₀	Aβ ₄₂	Aβ _{1-x}	Aβ ₄₀	Aβ ₄₂
ISF-hippo (pg/ml)	727.7 ± 92.1	224.4 ± 23.3	93.6 ± 16.9	626.4 ± 93.9	345.9 ± 52.2	73.9 ± 38.9
ISF-striatum (pg/ml)	207.1 ± 31.8			142.1 ± 19.5		
CSF (pg/ml)	11337 ± 699.7	5058 ± 496.0	2375 ± 336.8	8451 ± 915.3	4272 ± 898.7	961 ± 170.0
Hippo-carbonate (pg/mg protein)	255.0 ± 42.9	70.1 ± 14.5	80.3 ± 15.4	1147.6 ± 383.7	93.2 ± 29.7	692.5 ± 194.9
Hippo-guanidine (pg/mg protein)	1126 ± 135.9	105 ± 21.7	291 ± 30.4	87476 ± 29714	8159 ± 2775	66214 ± 22315

The concentration of ISF eAβ was assessed by microdialysis (n = 21). CSF was isolated from the cisterna magna and directly analyzed by ELISA (n = 6). Hippocampal tissues were processed by sequential extraction in carbonate buffer and then 5 M guanidine (n = 6). Tissue Aβ values are normalized to protein concentrations for each sample. Hippo, Hippocampus.

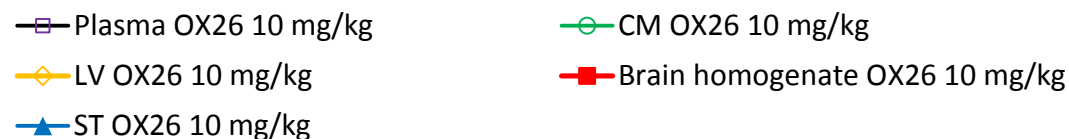
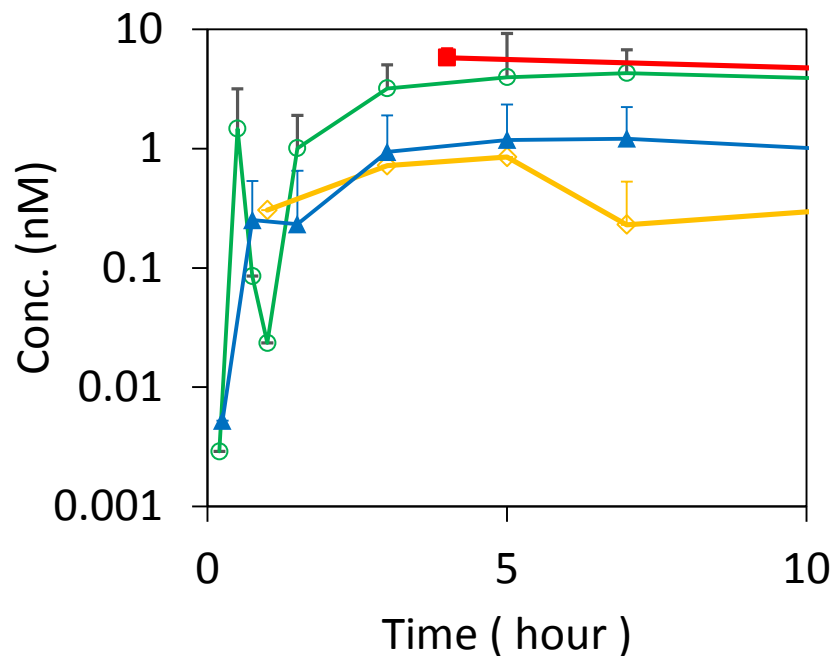
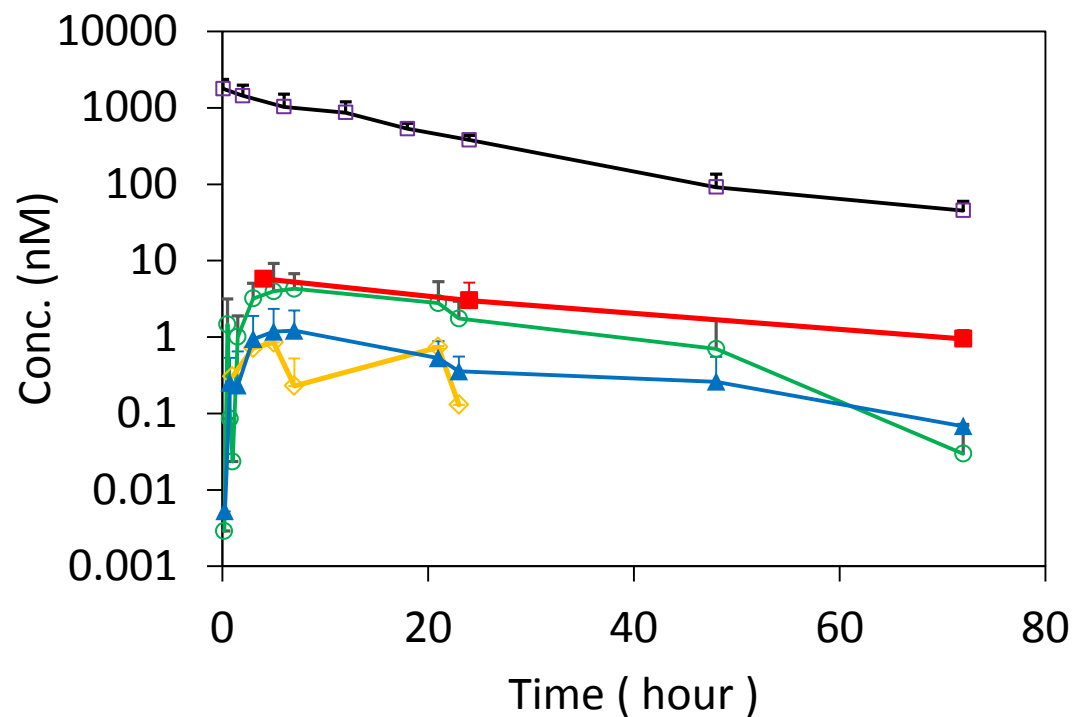
Brain Disposition of Trastuzumab



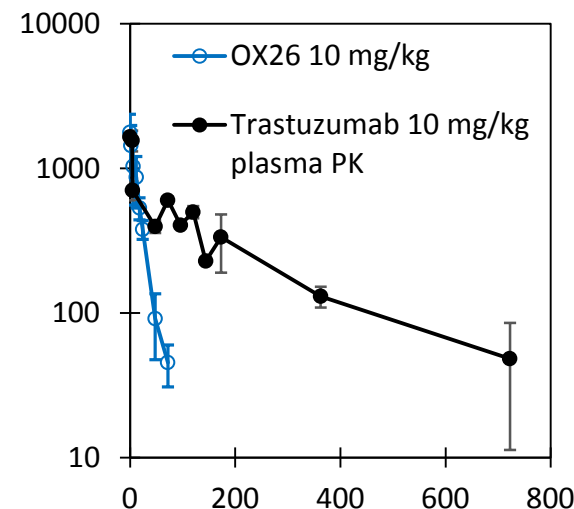
- Lag time for mAb distribution to striatum and CM
- Initially ST shows lower concentration than LV
- BCSFB may be an important contributor to delivery of mAb into the CSF at initial time point
- CM shows higher exposure similar to endogenous Ab



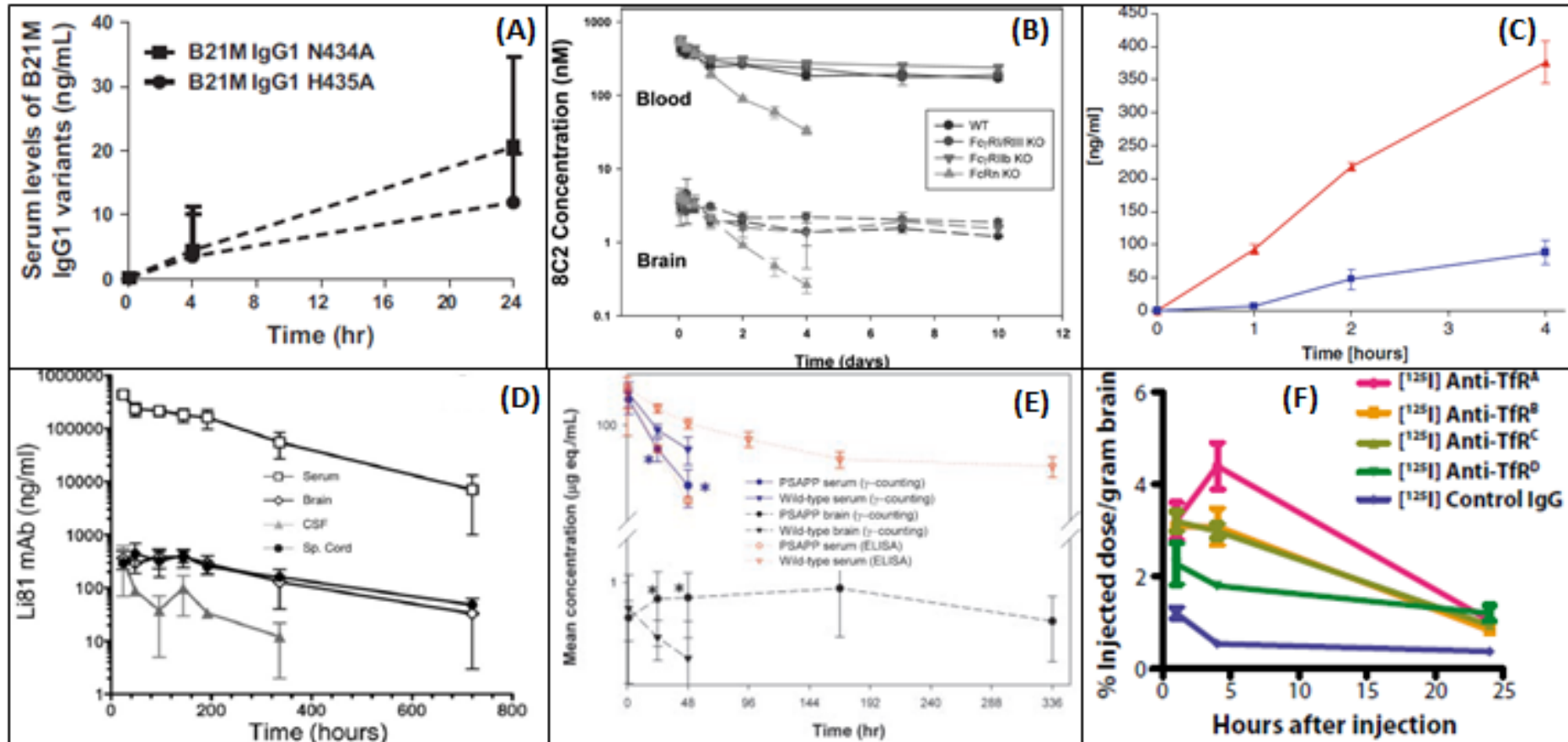
Brain Disposition of Anti-TfR Mab: OX26



- While brain homogenate concentration in high ST concentration is very low
- CM concentration is higher than ST and LV



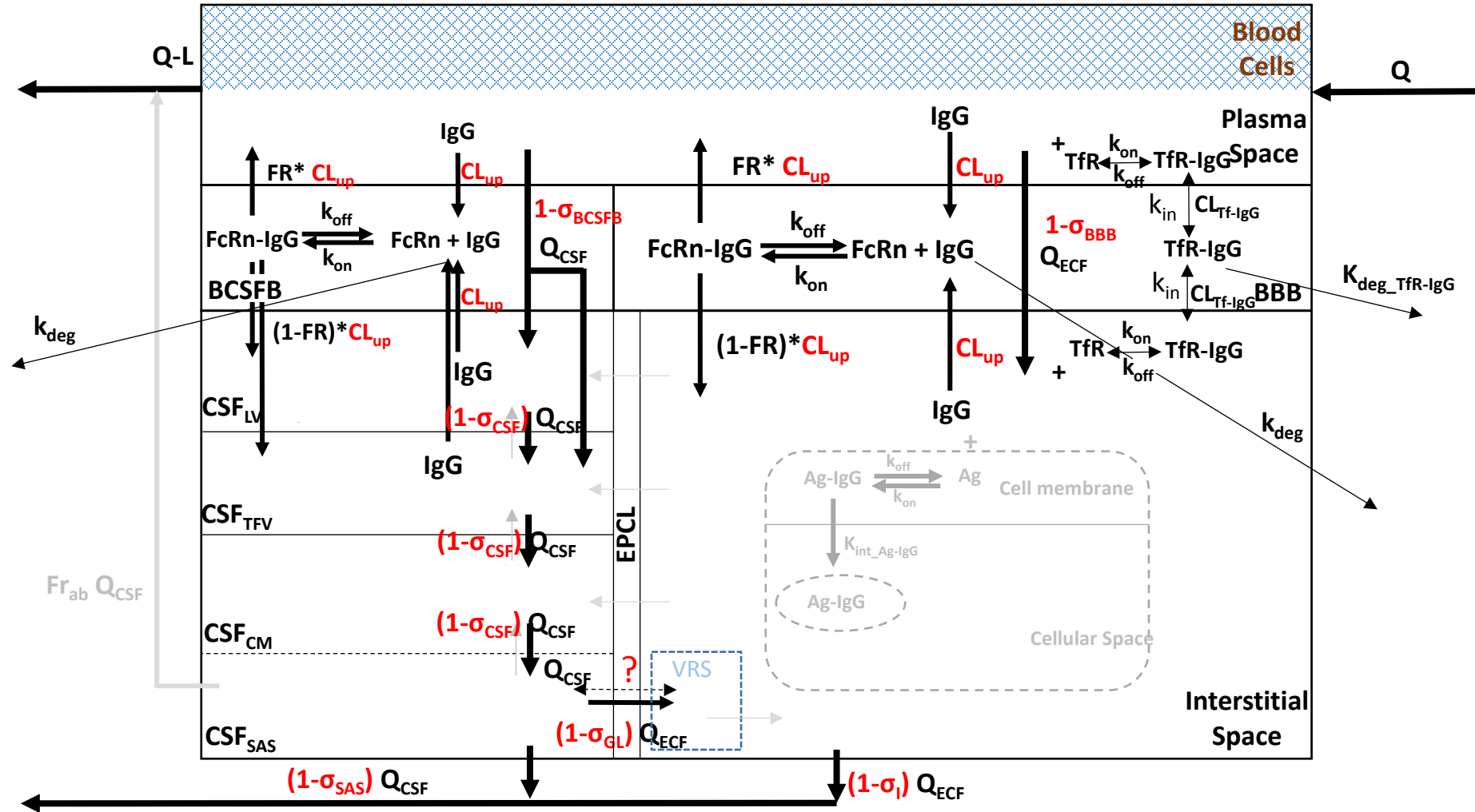
External Datasets



Diverse datasets to be used for building brain PBPK Model:

(A) Efflux of antibody from rat brain, (B) Antibody PK on FcRn KO mice, (C) Abluminal-to-luminal (red) and luminal-to-abluminal (blue) transport of Fc domain, (D) PK of antibody in different regions of rat brain, (E) PK of anti-A β antibody in the brain of antigen presenting PSAPP mice, (F) Brain uptake of different affinity anti-transferrin receptor antibodies.

PBPK Model for Brain Disposition of PTs

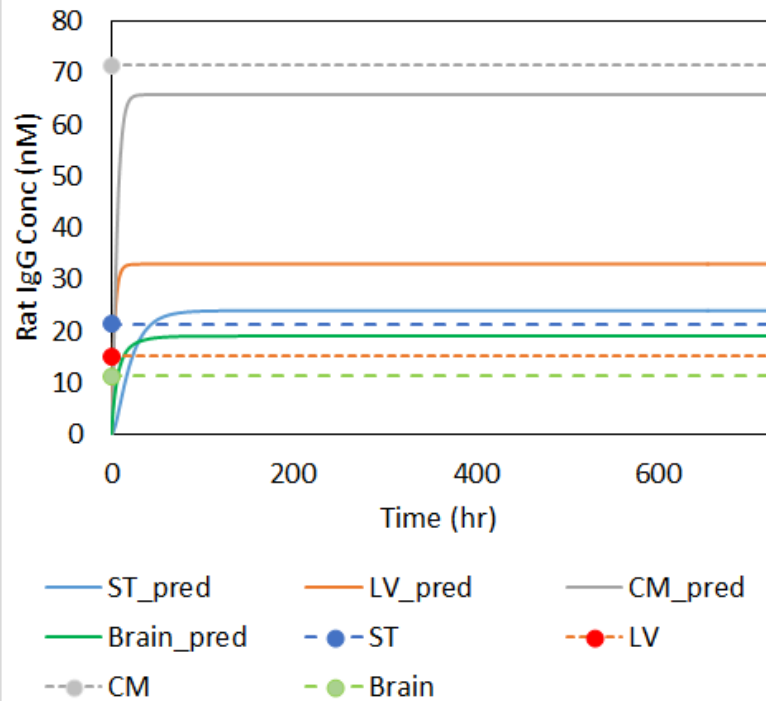


Parameters for Translational PBPK Model of Brain

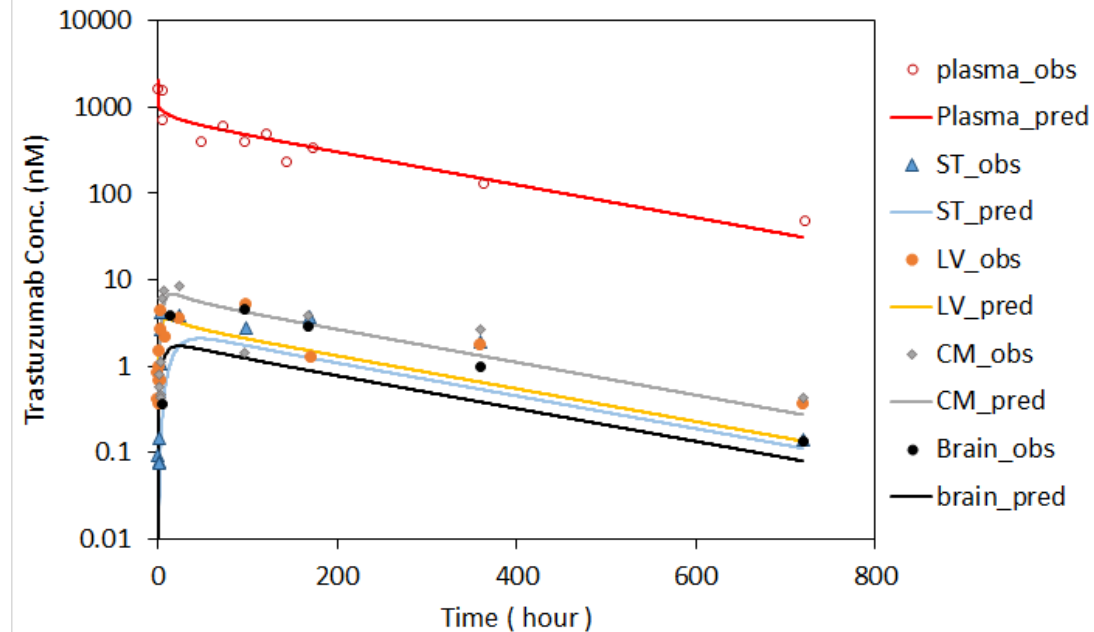
Values of brain physiological parameters in different species				
Parameter	Mice value	Rat value	Monkey value	Human value
CSF volume (ml)	0.035 ^[4] 0.04 ^[6]	0.25 ^[2]	13 ^[4]	150 ^[2] 100 ^[4] 160 ^[9] 150 -160 ^[10]
CSF production (ml/h)	0.0192 ^[4] 0.0198 ^[5]	0.132 ^[2]	2.46 ^[4]	21 ^[2] 24 ^[3] 24-36 ^[11]
CSF turnover (times/day)	13.333 ^[4]	11 ^[2]	4.615 ^[4]	4 ^[2] 5 ^[4]
Brain ECF volume (ml)	0.0406 ^b	0.29 ^[2]	26 ^b	240 ^[2]
Brain ECF production (ml/h)	0.00192 ^b	0.012-0.03 ^[2] 0.012 ^[3] 0.0216 ^[16] 0.02-0.31 ^[17]	1.23 ^b	9-12 ^[2] 10.5 ^[3]
Brain total volume (ml)	0.485 ^[1] 0.303 ^[7]	2.28 ^[1]	94.0 ^[1]	1450 ^[1]
Plasma volume (ml)	0.0107 ^[1]	0.0502 ^[1]	2.07 ^[1]	31.9 ^[1]
Blood cell volume (ml)	0.00873 ^[1]	0.0410 ^[1]	1.69 ^[1]	26.1 ^[1]
Interstitial volume (ml)	0.0873 ^[1]	0.410 ^[1] 0.29 ^[3]	16.9 ^[1]	261 ^[1] 240 ^[3]
Endosomal volume (ml)	0.00243 ^[1]	0.0114 ^[1]	0.470 ^[1]	7.25 ^[1]
Cellular volume (ml)	0.376 ^[1]	1.77 ^[1] 1.44 ^[3]	72.9 ^[1]	1124 ^[1] 960 ^[3]
Lateral ventricle volume (ml)	0.0039-0.0041 ^[8]	0.05 ^[3]	1.463 ^b	22.5 ^[3]
Third-Forth ventricle volume (ml)	0.0024-0.0025 ^[8]	0.05 ^[3]	1.463 ^b	22.5 ^[3]
Cisterna magna volume (ml)	0.0011 ^b	0.017 ^[3]	0.488 ^b	7.5 ^[3]
Subarachnoid space volume (ml)	0.0117 ^b	0.18 ^[3]	5.85 ^b	90 ^[3]
Surface area (meters ²)				
Brain		0.0140 ^[12] 0.0155 ^[12]		17 ^[4]
Choroid plexus		0.0025 ^[13]		1.7 ^[4]
Brain to CP ratio (surface area)	6.2 ^b	6.2		10 ^[4]
Brain weight (g)	0.3	1.8 ^[2]		1400 ^[2] 14
Cerebral blood flow (ml/h)	11.8 ^[1]	65.3 ^[1] 66 ^[2]	1508 ^[1]	21453 ^[1] 42000 ^[2] 63000 ^[4] 480 ^[4] 9 ^[9]
Choroid plexus blood flow (ml/h)				
Blood cell flow (ml/h)	9.64 ^[1]	53.5 ^[1]	1234 ^[1]	17553 ^[1]

PBPK Model Performance for Rat Data

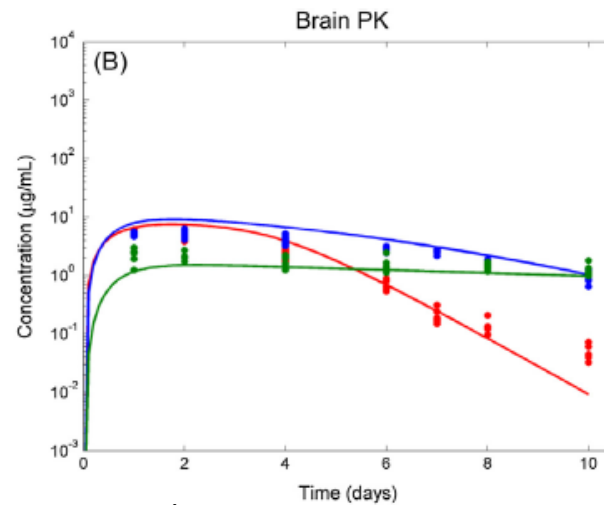
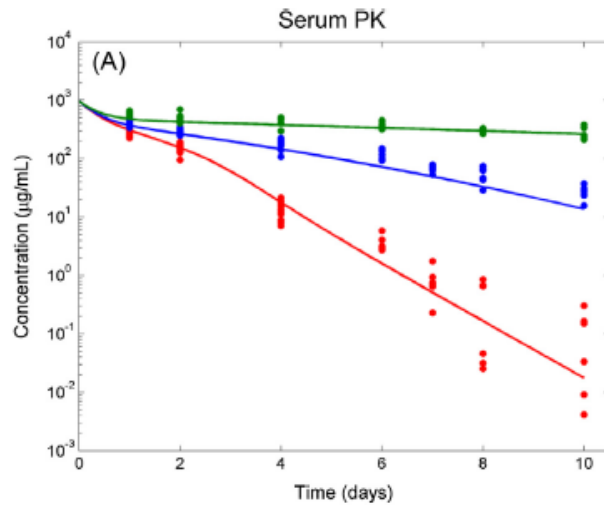
Fitting result of rat endogenous IgG



Trastuzumab disposition in rat brain



PBPK Model Performance for Anti-TfR MAbs in Mouse



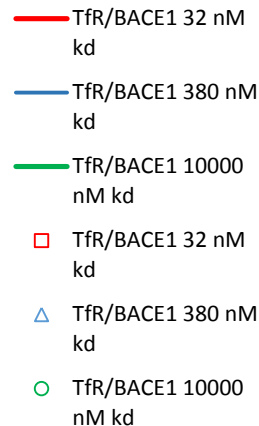
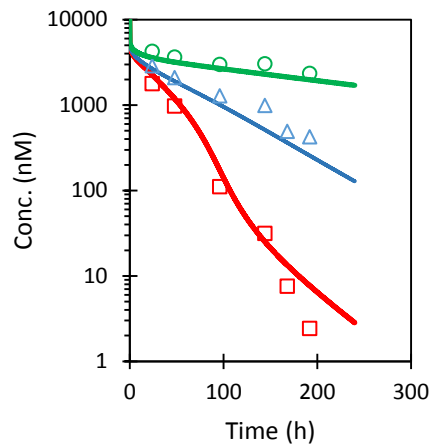
Anti TfR^A /BACE1, $K_d=32\text{nM}$

AntiTfR^D /BACE1, $K_d=380\text{nM}$

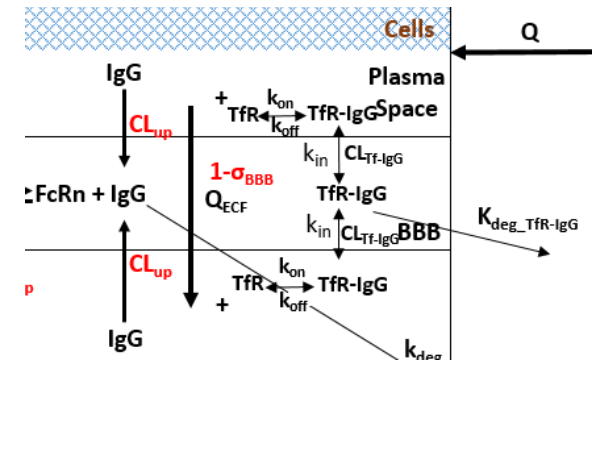
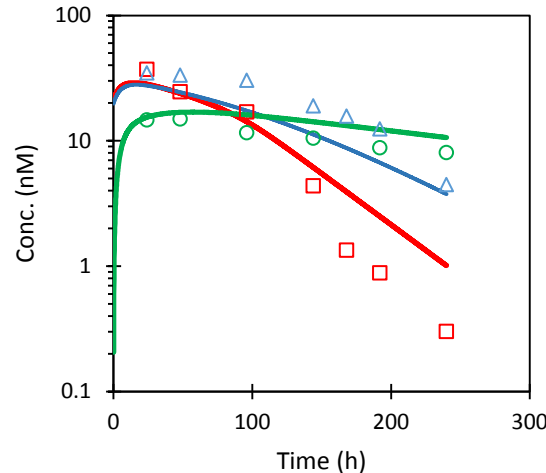
AntiTfR^E /BACE1, $K_d=10000\text{nM}$

	Final Estimate	CV%
V_TfR	910 nM	10.85
Kdeg_TfR	0.06 1/h	7.140
Fr_TfR	0.5	Fixed
Kint_B_TfR	7 1/h	Fixed
CL_TfR	18.00 L/h	Fixed

TfR/BACE1 Plasma PK



TfR/BACE1 Brain Homogenate PK



Concluding Remarks

- o In vivo microdialysis system and sensitive ELISA methods were developed to enable quantification of antibodies in different regions of brain
 - BCSFB may contribute notably towards CSF antibody concentrations
 - CM seems to have higher antibody concentrations than LV & ST
 - CSF concentrations of mAbs can be notably different than ECF
- o There is a need to generate more robust data using microdialysis and other orthogonal methods, and using different engineered proteins, to get a better understanding of protein therapeutics disposition in brain
- o A translational PBPK model is developed that is able to characterize the disposition of various antibodies in mice and rat; however more data is needed to refine the model

Acknowledgements



Center for Protein Therapeutics

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